The guinea pig as alternative species in developmental (and reproductive) toxicology studies

Steffen Schneider, BASF SE

Outline

- Why guinea pig ?
- Biology of reproduction (basics)
- Study design
- Problems, Pitfalls
- Preliminary data, procedural experience
- Resume

Why guinea pig ?

Alternative Model when testing in standard models is not appropriate, i.e. when

- **test item pharmacology, kinetics and metabolism require an alternative species**
- **special questions of hormonal regulation of pregnancy maintenance and parturition or of placentation need to be adressed**

Literature data assume that

- hormonal regulation of pregnancy and parturition are more similar to humans than rat and rabbit
- guinea pigs are sensitive to teratogenic effects
- it is a good alternative rodent model for human placentation

Biology of Reproduction

BASF
The Chemical Company

Physiological milestones

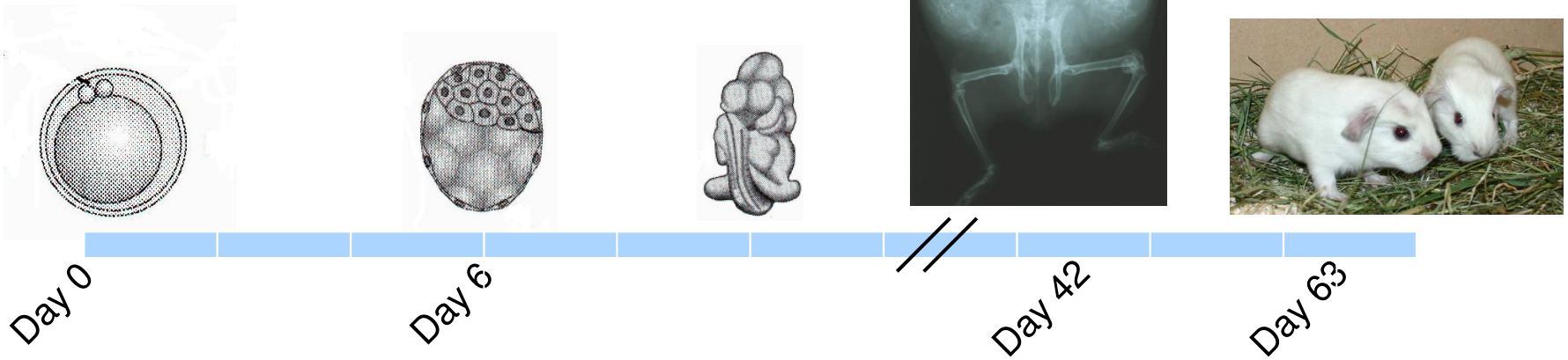
- Lifespan 4 years
- Sexual maturity: males 3-4 months (600-700 g), females 2-3 months (350-450g)
- completely developed at birth (complete fur, open eyes, teeth, eat adult diet from PND 1)
- Weight at weaning 150-200g

Biology of Reproduction

Milestones of reproduction

	Guinea Pig	Rat	Rabbit
Strain	**Dunkin-Hartley**	**Wistar Han**	**New Zealand White**
Estrous cycle	**15-17 days**	**4-5 days**	**28 days**
Estrous duration	**6-10 hours**	**10-18 hours**	**Provoked ovulation**
Duration of gestation	**64-70 days**	**22-23 days**	**30-31 days**
End of embryonic phase (closure of hard palate)	**GD 42**	**GD 15**	**GD 19**
Litter size (average)	**4**	**12**	**8**
Birth weight	**60-80 g**	**5-6 g**	**35-40 g**
Lactation	**14-28 days**	**21 days**	**42 days**

Study design



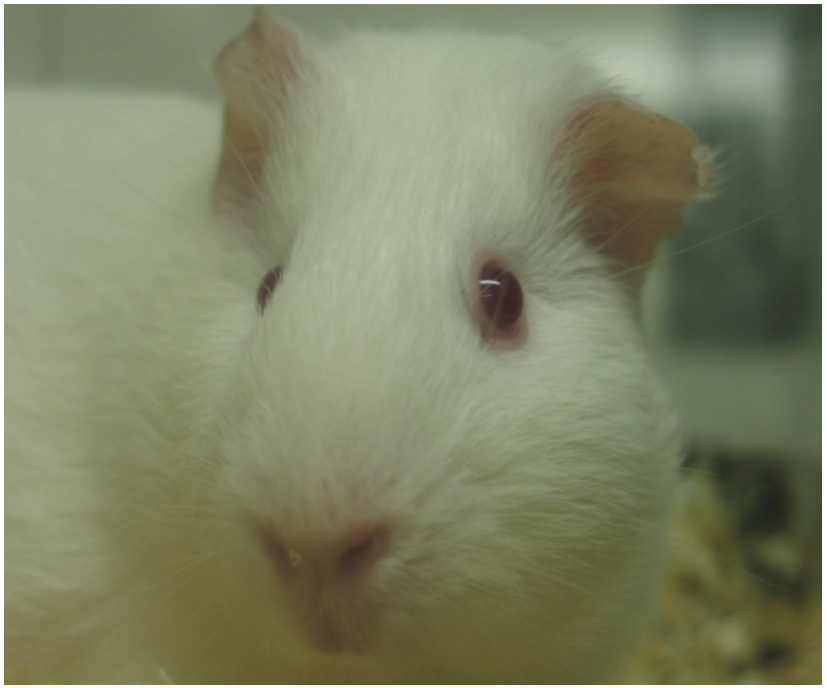
Administration period

Prenatal Developmental Toxicity Study
Guinea Pig

BASF
The Chemical Company

Pregnant Females

- Dunkin-Hartley (outbred albino strain)
- N= about 20 litters / group (start with 30)
- Treatment duration GD 6 through 62
- C-Section on GD 63



Prenatal Developmental Toxicity Study
Guinea Pig

BASF
The Chemical Company

Inlife parameters

- Mortality, clinobs daily
- Food consumption, body weights twice weekly
- Any other examinations/parameters to characterize maternal effects on a case by case basis
- In our study: hematology, clinical chemistry, steroid hormones on GD 63

Prenatal Developmental Toxicity Study
Guinea Pig

BASF
The Chemical Company

C-section parameters

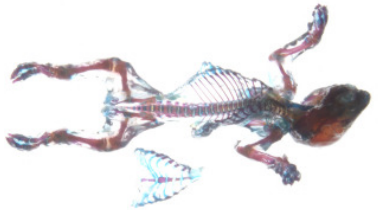
- Uterus weight
- Ovary weight
- Number of Corpora lutea
- Implants, resorptions, live/dead fetuses
- Weight and preservation of placenta
- Other targets identified in rangefinding studies …

Prenatal Developmental Toxicity Study Guinea Pig

Fetal parameters

- Number and weight
- Sex distribution
- Fetal pathology

- External
  - Malformations
  - Variations
  - Undefined observations
- Soft tissues
  - Malformations
  - Variations
  - Undefined observations
- Skeletons
  - Malformations
  - Variations
  - Undefined observations



Practical Considerations

BASF
The Chemical Company

Problems / Pitfalls

- How to get appropriate numbers of time-mated females within a decent timeframe ?
- Dosing and anesthesia problems
- Adaptation of double staining method to a mature skeleton
- Little detailed information on developmental milestones, morphology etc. available
- No historical data (C-section, external, visceral, skeletal)

Practical Considerations

Generation of time-mated females, solution 1

- Purchase time-mated females from Charles River
- Mated overnight on postpartal estrous
- Reasonable timeframe – 20 pregnants per week

- No primipara
- Heterogeneous in terms of age and numbers of litters they already had (1-5)
- Delivery on GD 4 - very short adaptation time before treatment

Preliminary Results

BASF
The Chemical Company

Cesarean data

Parameter	Range Dunkin Hartley GP	Range Wistar Han Rat
Conception rate	77 – 90%	93%
Abortions	0 – 10%	0
Pregnant at C-Section	63 – 77%	93%
Corpora Lutea	5.6 – 5.8	9.2 – 11.3
Implants	4.4 – 4.8	7.7 – 10.3
Pre-implantation loss	16.4 – 21.4%	3.2 – 19.6%
Post-imlantation loss	8.8 – 14.3%	2.7 – 14.0%
Early resorptions	7.2 – 13.2%	
Late resorptions	0.0 – 2.4%	
Dead fetuses	0	0

Preliminary Results

BASF
The Chemical Company

External / Visceral examinations

- External examination similar to external inspection during necropsy in adult animals
- Full grown fur may mask subtle findings, e.g. small meningocele
- Visceral examination similar to rabbit examination

- In our study no spectacular findings

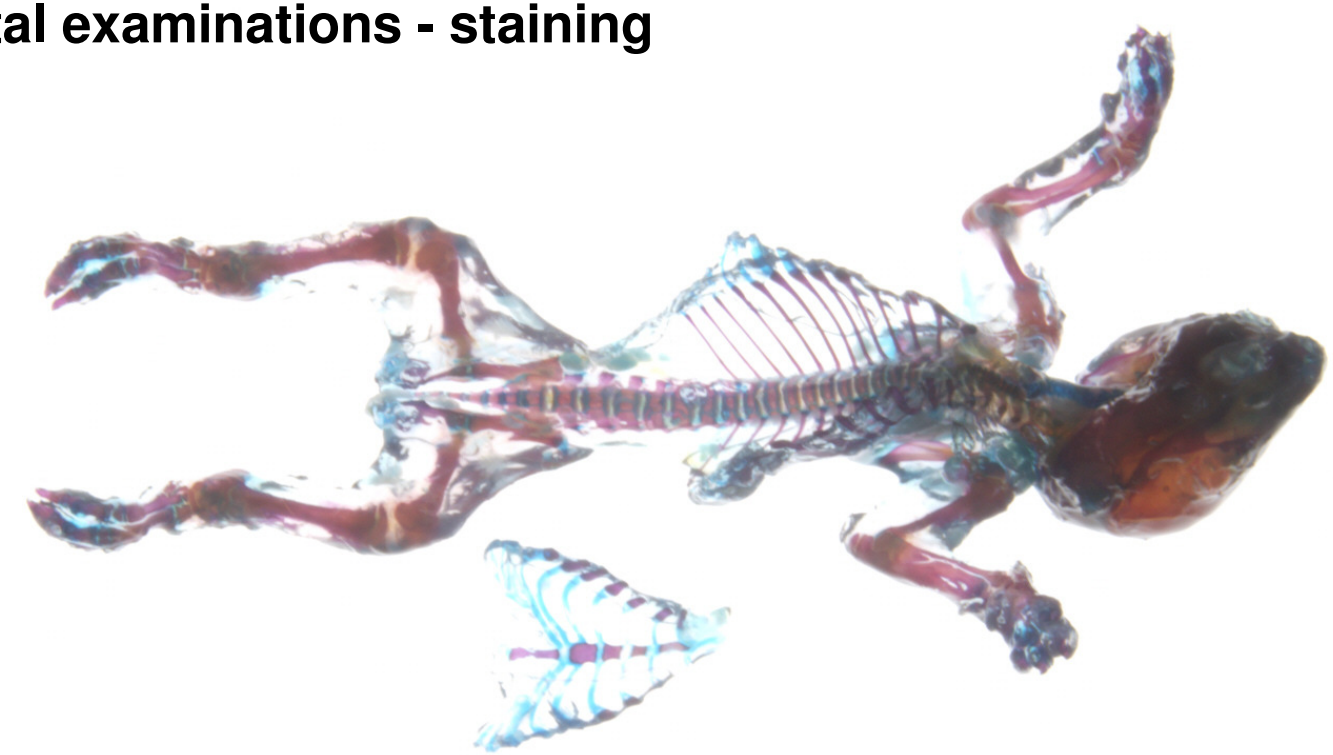
Preliminary Results

Skeletal examinations

- Double staining adapted to GP skeleton, rabbit procedure didn´t work properly
- Skeletons considerably bigger than rabbit skeletons – need bigger processing vessels and more space in archive
- Removal of skin and adjacent tissue particularly important in skull area
- Limbs / phalanges robust against „skinning damage“
- When heads severed from trunk for Wilson examination the 1st cervical vertebra always goes with the head
- Incise skull bones without damage to inspect brain and for better penetration of KOH difficult, because completely ossified and hard
- Best information source: P. Popesko et al., Colour atlas of the anatomy of small laboratory animals, Vol 1, rabbit and guinea pig, Wolfe Publishing Ltd., 1992

Preliminary Results

Skeletal examinations - staining



Preliminary Results

Skeletal examinations - staining

Preliminary Results

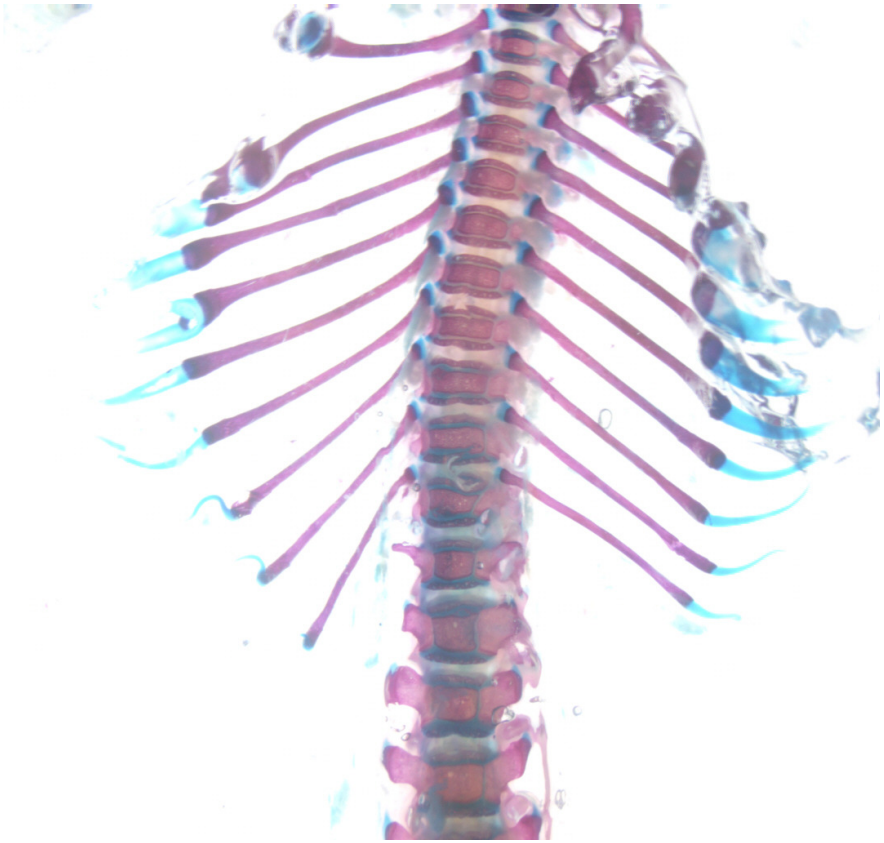
Skeletal examination – selection of control data

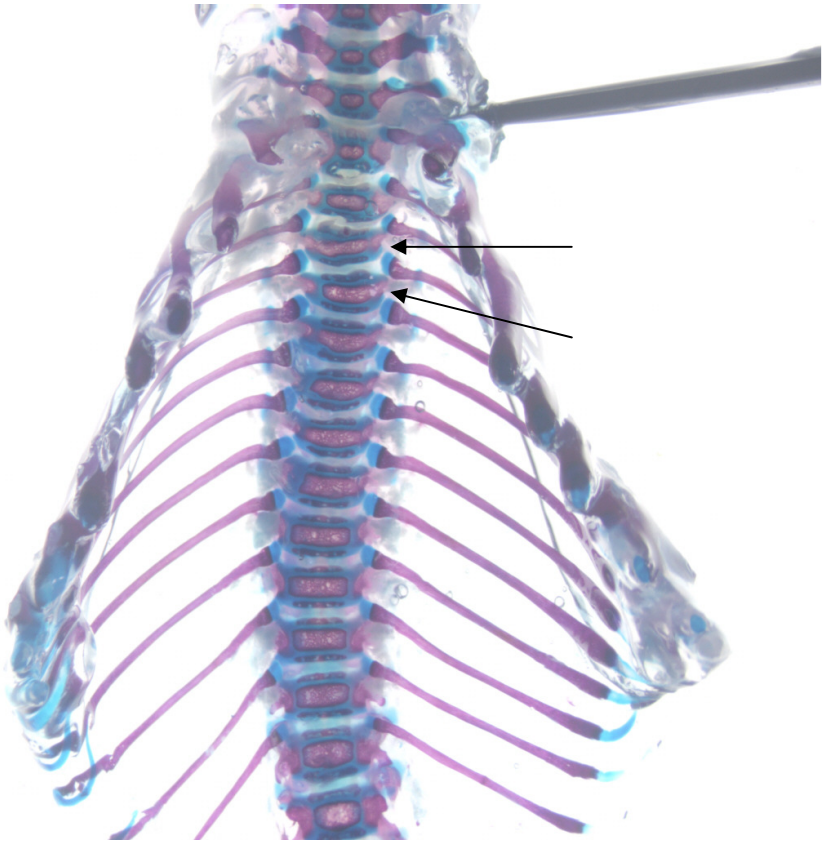
Structure	Findings
Skull	9.25% with incomplete ossification of hyoid
Forelimbs, Hindlimbs, Pelvis	No findings
Sternum	83.5% with 5 sternebrae 16.5% with 6 sternebrae, 6th sternebra usually rudimentary
Rib cartilages	96% with 6 rib cartilages connected to sternum 4% with 7 rib cartilages connected to sternum 1% with fused rib cartilage 1% with displaced rib cartilage
Vertebrae	100% with 7 cervical, 13 thoracal and 4 sacral vertebrae 6.3% with 5 lumbar vertebrae 92.7% with 6 lumbar vertebrae 1% with 7 lumbar vertebrae
Ribs	82% with complete, unchanged 13 rib pairs 16% with small (rudimentary) 13th 2% with 14 rib pairs, usually small (rudimentary) 14th

Preliminary Results

BASF
The Chemical Company

Skeletal examinations - findings

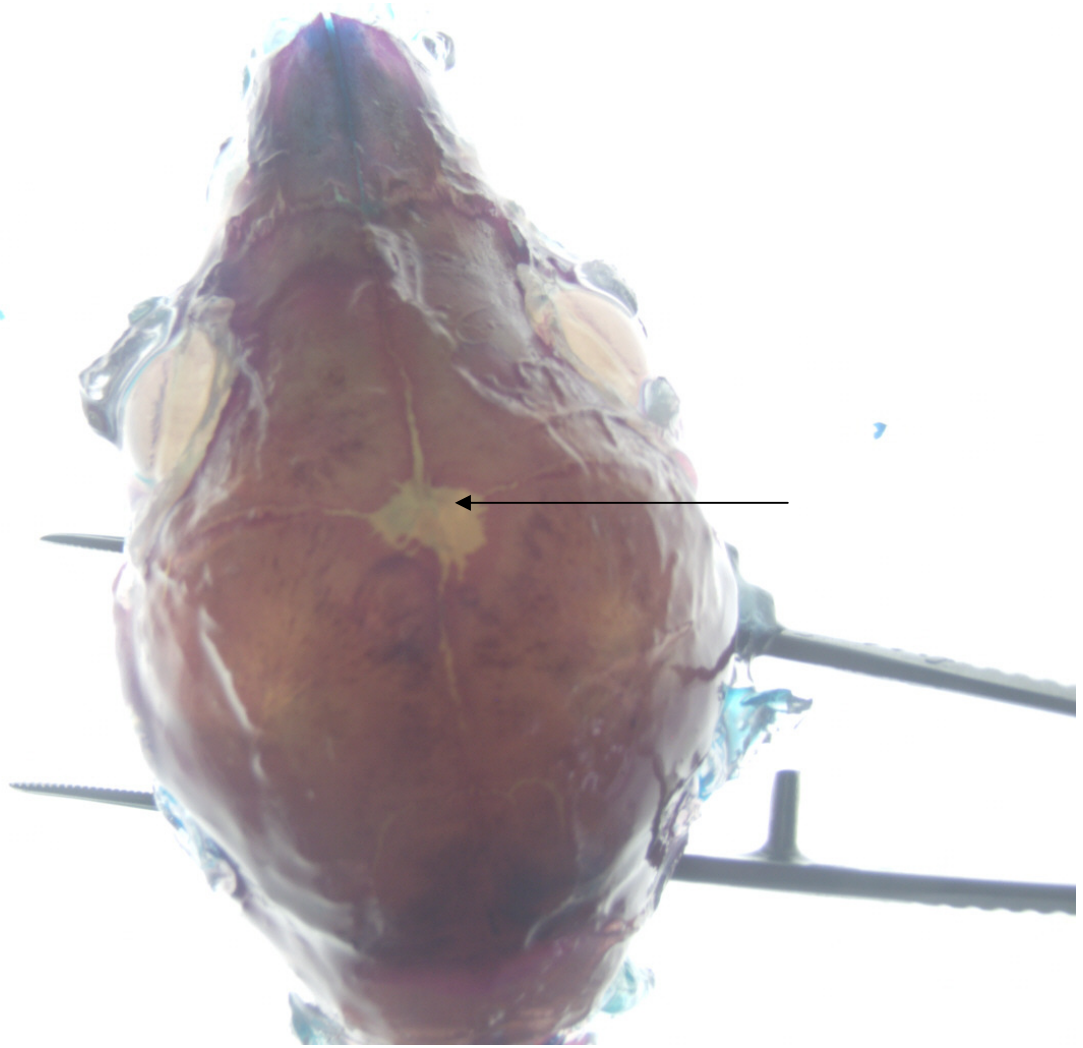




Preliminary Results

BASF
The Chemical Company

Skeletal examinations - findings

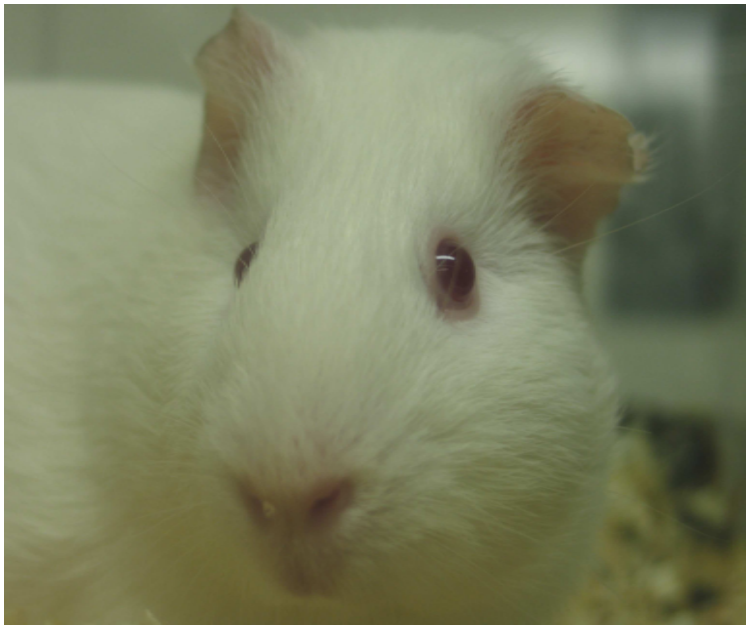


Pre-Postnatal Reproductive Toxicity Study Guinea Pig

BASF
The Chemical Company

Pregnant Females

- N= 25 litters / group (start with 30 to ensure enough litters)
- Treatment duration GD 6 through PND 21
- Weaning/study termination on PND 21
- Primipara required, because number of parturitions influences the duration of pregnancy



Practical Considerations

BASF
The Chemical Company

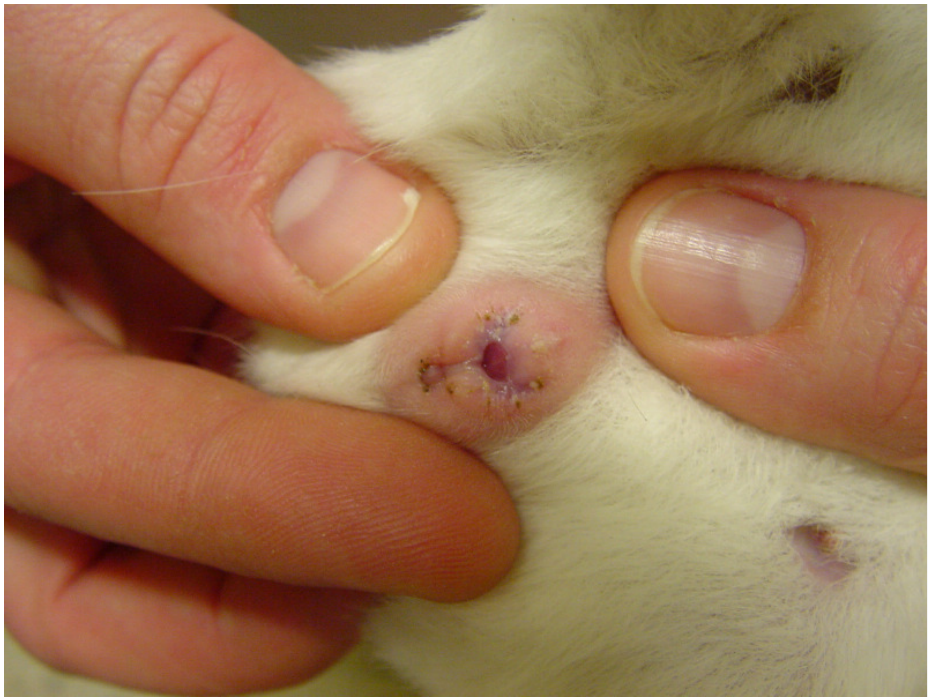
Generation of time-mated females, solution 2

- Mate females in the lab
- No reasonable timeframe possible because
  - long estrous cycle – if one estrous is missed for pairing, it takes almost 3 week until next chance
- Estrous is easily missed
  - no daily vaginal smear/lavage possible to check cyclic changes of vaginal epithelium because of vaginal membrane
  - vaginal lavage after overnight pairing to check for sperm gives uncertain results because sperm rapidly resorbed and often not present anymore in the morning
- Pairing after opening of vaginal membrane without sperm check produces a 50% rate of non-pregnant females

Practical Considerations

Generation of time-mated females, solution 2

- Check for open vaginal membrane twice each day



Practical Considerations

BASF
The Chemical Company

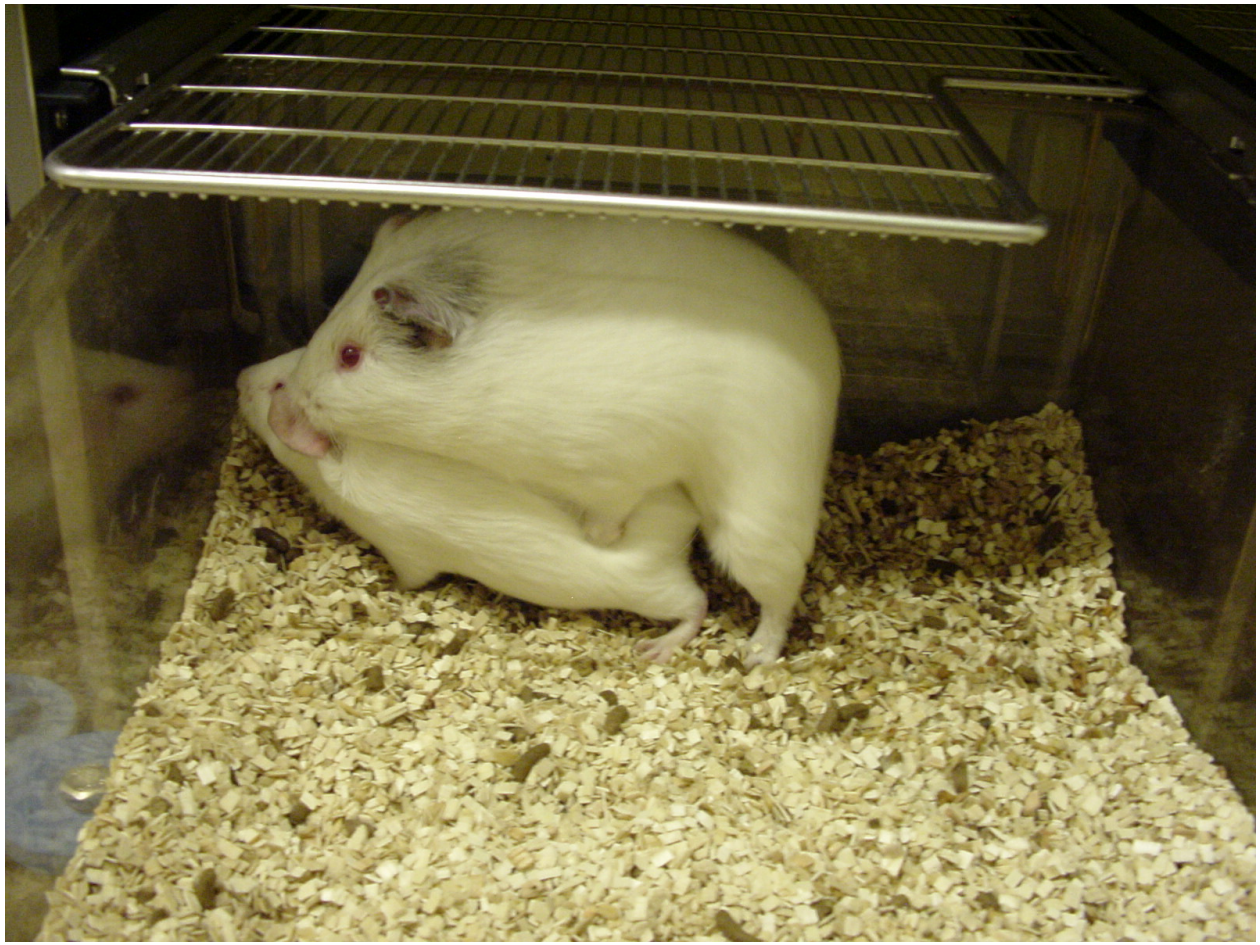
Generation of time-mated females, solution 2

- Check for open vaginal membrane twice each day
- When membrane open
    - take vaginal smear/lavage
- When smear shows estrous (only exactly estrous)
    - put female to a male

Practical Considerations

Generation of time-mated females, solution 2

- Observe pairing
- Look for proper mountings
- Palpate for presence of offspring after (about) 25 days



Practical Considerations

BASF
The Chemical Company

Generation of time-mated females, solution 2

- Pregnancy rate 100%
- Still almost 3 months to get 100 timed-pregnant females
- get hundreds of these after 68-69 days

Resume

- There are disadvantages / problems
  - difficult to get time-mated primipara
  - long duration of gestation
  - small litter size
- Little experimental experience for repro testing – gather experimental details the long way
- Collection of historical data takes ages
- Labs with sufficient experience can produce reliable data
- Remains a model for exceptional cases

Ideal model

Guinea rat